

THE SUBTERRANEAN INTERMEDIARY ORGANS OF *DIOSCOREA COTINIFOLIA* KUNTH: 1. THE GERMINATION, DEVELOPMENT, MORPHOLOGY AND VEGETATIVE REPRODUCTION OF THE TUBEROUS SWOLLEN AND CYLINDRICAL INTERMEDIARY ORGANS.

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ABSTRACT

Germination and morphological studies were carried out on several Transvaal *Dioscorea* species. It is concluded that since (1) the number of vascular traces entering the petiole of the cotyledon differs from that entering the first vegetative leaf and (2) the venation of the cotyledonary lamina also differs from that of the first leaf, the embryo can have only one cotyledon. *Dioscorea cotinifolia* has a specialised tuber system consisting of a crown and several tubers borne by long, cylindrical organs. All these organs, which originate directly or indirectly by tuberisation and/or branching from the hypocotyl, are regarded as intermediary organs. These intermediary organs have characteristics typical of both the shoot and the root. They develop from a deep-seated lateral meristem found neither in the shoots nor the roots of *Dioscorea*. The intermediary organs initially serve as absorptive organs and bear adventitious roots. The main functions of the intermediary organs are those of storage and vegetative reproduction.

UITTREKSEL

DIE ONDERGRONDSE INTERMEDIËRE ORGANE VAN *DIOSCOREA COTINIFOLIA* KUNTH. DIE ONTKIEMING, ONTWIKKELING, MORFOLOGIE EN VEGETATIEWE VOORTPLANTING VAN DIE KNOL, KNOLVORMIGE EN SILINDRIESE INTERMEDIËREORGANE.

Die saadontkieming en morfologie van verskeie Transvaalse *Dioscorea*-soorte is ondersoek. Aangesien die aantal vaatstringe wat die blaarsteel van die saadlob binnegaan verskil van dié wat die eerste vegetatiewe blaar binnegaan en aangesien die bearing van die saadloblamina verskil van dié van die eerste blaar, is afgelei dat die embrio oor 'n enkele saadlob beskik. *Dioscorea cotinifolia* het 'n gespesialiseerde knolsisteem, bestaande uit die kroon en verskeie knolle wat aan lang silindriese organe gedra word. Al hierdie organe, wat direk of indirek deur knolvorming en/of vertakking uit die hipokotiel ontstaan, word as intermedieëre organe beskou. Hierdie intermedieëre organe besit stingel- sowel as wortelkenmerke. Hulle ontstaan uit 'n diepgeleë laterale meristeem wat nóg in stingels nóg in wortels van *Dioscorea* aangetref word. Die intermedieëre organe dien aanvanklik as absorpsiorgane. Hulle dra bywortels, help om die plant te anker en vervul veral die funksie van stoor en vegetatiewe voortplanting.

1 INTRODUCTION

The Dioscoreaceae was first recognised as a family in 1819 (Burkill, 1960) and, according to Bucherer (1889), Dutrochet as early as 1835 commented on the

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embryonic cotyledons and the peculiar growth of the tuber of *Tamus communis*, a genus closely related to *Dioscorea*. It was De Barry (1884) who distinguished three types of storage organs in this family: (1) tuberous swollen roots; (2) rhizomes; and (3) leafless tubers resulting from the swelling of the first epicotyledonary internode of the seedling. He stated, however, that tubers "require more exact investigation in all points". Goebel (1905), probably following the opinion of earlier workers including Queva (1894) and Leclerc du Sablon (1902) as quoted by Burkill (1960), described some Dioscoreaceae tubers as "Organe, die ohne aus einer Umbildung von Wurzeln oder Sprossen hervorgegangen zu sein, in ihren Eigenschaften teils den Sprossen, teils den Wurzeln nahestehen".

Burkill (1960) himself considered the tuber to be "a contraction from the rhizome", i.e. a stem structure.

Koch and Bruhn (1962) regarded the tuber of *D. floribunda* as a thickening of the hypocotyl. Martin and Ortiz (1963) although confirming many of Koch and Bruhn's observations were of the opinion that the tubers of *D. floribunda* and *D. spiculiflora* comprised "much modified stem tissue, thus being a relic of the ancestral rhizome". Troll (1967), apparently basing his opinion only on the observations of other workers, rejected Goebel's opinion, maintaining that it could be demonstrated incontestably that the tubers of *Tamus communis*, *Dioscorea sinuata* and *Testudinaria elephantipes* were shoot tubers.

Ayensu (1972) concluded that further studies were necessary to understand the true morphological nature of the storage organs. He also reviewed the "controversy in the literature concerning the number of cotyledons". Two recent publications showed that the number of cotyledons is still in dispute (Sharma, 1974; Sadik and Okereke, 1975).

The results of a detailed morphological study of the seven *Dioscorea* species occurring in the Transvaal (Republic of South Africa), which included a germination study of four species, have already been partly described (von Teichman und Logischen, 1973; von Teichman und Logischen, *et al.*, 1975). This paper reports on the seed germination, development and morphology of the tuber system and vegetative reproduction of *D. cotinifolia*. An account of the anatomy of the aerial shoot and the root in comparison with the tuber system of *D. cotinifolia* is in preparation.

2 MATERIAL AND METHODS

2.1 General

Observations were made from specimens in the National Herbarium in Pretoria, the Moss Herbarium of the University of the Witwatersrand, Johannesburg, the Herbarium of the University of Pretoria, and from personal records.

Material, involving the removal of one or more entire plants, was collected at 20 different localities in the Transvaal. At least one herbarium specimen was

prepared from every plant while some tubers were planted in the garden of the University of Pretoria and others preserved in formalin-acetic acid-alcohol (FAA) together with remainders of the shoots, after material had been reserved for anatomical studies.

2.2 Germination

After preliminary germination tests the following optimal treatment was used: seeds were soaked in tap water for 40 to 48 h, surface-sterilised for 1 min with undiluted commercial bleach and rinsed five times with sterile tap water. The seeds were either placed in sterilised vermiculite in pots or on sterilised filter paper in petri dishes. Sterilised tap water was applied.

Seeds were germinated in the dark at 27° C in a room with a relative humidity (R.H.) of 70%. After the radicle and first leaf had appeared the seedlings on the filter paper were transferred to vermiculite. All seedlings were kept at 27° C and 70% R.H. under constant light for one month and a nutrient solution (see APPENDIX), was applied three times every week. Seedlings were then transferred to sandy soil and kept in a glasshouse for two months at temperatures and relative humidities with average maxima and minima of 35 and 18° C and 96 and 70%, respectively.

Seedlings were cleared in 85% lactic acid at 60° C, washed with distilled water and stained in 0.1% basic fuchsin (aqueous) for three hours; excess stain was removed with distilled water followed by 70% ethanol. The cleared seedlings were then transferred back to lactic acid and examined and dissected in glycerine.

3 OBSERVATIONS AND DISCUSSION

As Archibald (1967) published a detailed morphological account on *D. cotinifolia* from the Cape Province west of East London, only a few additional remarks are necessary. In the Transvaal *D. cotinifolia* commences flowering in October, the trilocular capsules starting to ripen in about January. The distally winged seeds (Fig. 1A) contain a 1.16 to 1.25 mm long embryo which lies loose in the slit of the predominantly proteinaceous endosperm. Dissection of seeds was easy since the embryo is situated near the raphe, opposite a small dark patch in the adjoining wing (Fig. 1B). This patch appears darker because of the heavy pigmentation of the two inner layers of the wing. In FAA-preserved and -soaked seeds the raphe containing the vascular tissue could be pulled off together with a little bit of the surrounding tissue. The position of the embryo in the seed is constant in all the species within a section (Fig. 2, 3 and 4 in von Teichman und Logischen *et al.*, 1975).

The cotyledon forms a one-lobed (Fig. 1C) or two-lobed, very inconspicuous flap (Fig. 1D) which conceals the plumule partly or wholly. When this cotyledonary sheath is removed, the first vegetative leaf primordium can be distinguished. The growing point of the stem can be seen only in longitudinal sections (Fig. 1E).

The embryos of the seven species studied show much resemblance to one another and to that of *D. bonariensis* illustrated by Beccari (1870). Beccari, however, interpreted part of the cotyledonary sheath as the second rudimentary cotyledon. On the other hand the embryo of *D. villosa* (Smith, 1916) differs in having no cotyledonary sheath. The embryos described by Rao (1953), Lawton and Lawton (1967), Sadik and Okereke (1975) and others, also resemble that of *D. cotinifolia*.

3.1 Germination

Germination studies were done on *D. dregeana*, *D. quartiniana* and *D. sylvatica* in addition to those on *D. cotinifolia*. Only a few interesting differences are mentioned here.

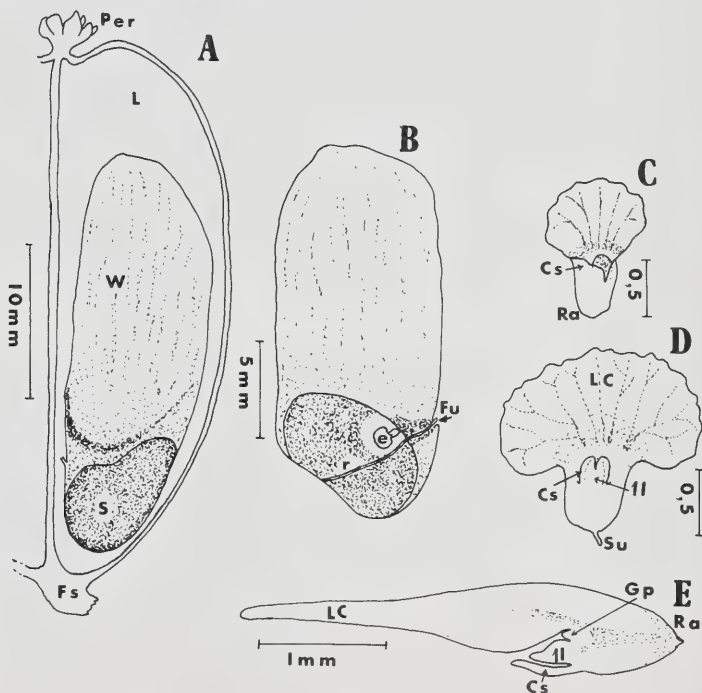


FIG. 1.

Morphology of the seed and embryo of *D. cotinifolia*. A: Position of seed in the locule; B: position of embryo in the seed; C & D: Embryos; E: Ls of embryo. Cs cotyledon sheath; e embryo; Fs fruit stalk; Fu funicle; Gp growing point of stem; L locule; LC lamina of cotyledon; Il first vegetative leaf; Per perianth; Ra radicle; r raphe; S seed; Su suspensor; W seed wing.

The seeds were considered to have germinated once the radicle had emerged (Fig. 3A). Germination of the fully ripened seeds of *D. cotinifolia* took place after an average of 11 days, the range being 9 to 12 days. About four days later the first vegetative leaf appeared still surrounded by the delicate sheath (Fig. 2B and 3B). In *D. sylvatica* the cotyledonary sheath becomes tube-like and has a distinct slit through which the first leaf emerges. This is similar to seedlings of *D. elephantipes*, a closely related species (Sparshott, 1935). The seedlings of *D. cotinifolia* illustrated in Fig. 3A and 3B were cleared, dissected and the complex course of the vascular tissue reconstructed (Fig. 2A and 2B). A large number of idioblasts containing mucilage and raphide bundles are present. On entering the cotyledon stalk (i.e. petiole), the six basal veins of the cotyledon lamina unite intricately (Fig. 2A). The two vascular traces from the cotyledon stalk (Fig. 2B:VTC) unite shortly before they enter the epicotyl-hypocotyl region while a single element (tracheid) branches off and re-unites. The origin of the three vascular traces of the first vegetative leaf (Fig. 2B:VT11) is clearly distinguishable. A single element branches off to join one of the cotyledonary traces (VTC). Two lateral root primordia are already developed. The first leaf is protected by the cotyledonary sheath.

Similarly, the primordium of the second leaf lies protected in the leaf sheath of the first (Fig. 4A). This is typical of monocotyledonous leaves. A large number of club-shaped multicellular glandular trichomes occur on the first leaf (Fig. 2B). In Fig. 2B the idioblasts were omitted from the drawing for the sake of clarity.

The lamina of the cotyledon which serves as an haustorium enlarges (Fig. 3C) until it occupies the whole slit in the endosperm. The lamina of the first leaf which unfolds about 16 days after germination is the largest of all the leaves on the primary stem. It is as large as or larger than the mature leaves of the parent plant. The venation of the first leaf (Fig. 3D) resembles that of mature leaves which also have seven main veins at the base. The veins of the cotyledonary lamina (Figs. 2 and 3C) on the other hand fuse to form three veins at the base.

In their review, Weber and Troll (1969) stated that the results of Lawton and Lawton (1967) deserved re-examination and that their illustration of the "second cotyledon" as a small "hump" in the embryo indicated that it could in fact be regarded as the first vegetative leaf. Recently Sharma (1974), apparently without much personal investigation, adopted the idea of Lawton and Lawton. Since the venation of the cotyledonary lamina differs from that of the first vegetative leaf and since there is a difference in the number of vascular traces in the petioles of these leaves, it was concluded that there is only one cotyledon in *Dioscorea*. This confirms the ideas of Sparshott (1935), whose serial transverse and longitudinal sections indicate *inter alia* the presence of a very short epicotyl. Lawton and Lawton's (1967) report on the presence of two cotyledons in *Dioscorea*, however, ignored the results of Sparshott.

The very first sign of a tuber, i.e. a swelling of the hypocotyl, appears

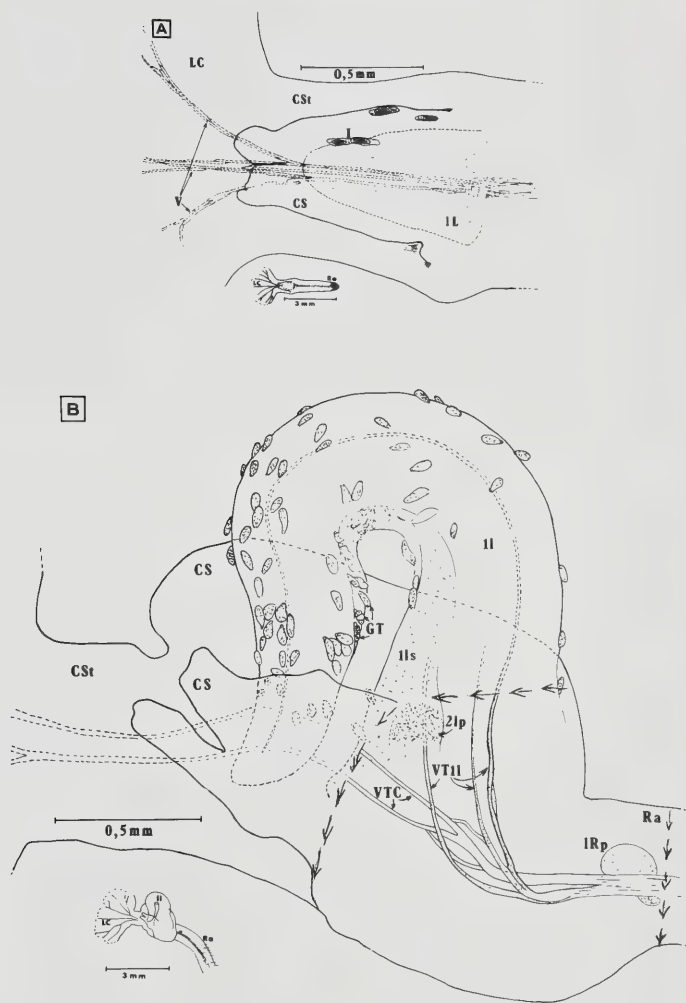


FIG. 2.

Young seedlings of *D. cotinifolia*, cleared and dissected. A: The seedling shown in Fig. 3A; B: The seedling shown in Fig. 3B. CS cotyledon sheath; CSt cotyledon stalk; GT glandular trichomes; I idioblasts; LC cotyledon lamina; IL/II first vegetative leaf; 2lp second leaf primordium; 1ls first vegetative leaf sheath; IRp lateral root primordium; Ra radicle; VTC vascular traces of cotyledon; VT1l vascular traces of first vegetative leaf; V veins. In the region demarcated by arrows the uppermost tissues were removed.

simultaneously with the expansion of the first leaf lamina (Fig. 3C). Before or as the second leaf appears, approximately 26 days after germination, the tuber is usually easily distinguishable directly below the first leaf base (Fig. 3E, F, G and H). The tuber therefore originates in the hypocotyl, confirming the reports of many other workers.

The main root is pushed to one side by the enlarging tuber. Lateral and adventitious roots develop from the main root and young tuber, respectively, at a very early stage (Figs. 3C and 3E).

The growing point of the primary stem can be distinguished at the base of the second leaf sheath. In *D. cotinifolia* the stem developed about 37 days after germination. In *D. sylvatica*, *D. quartiniana* and *D. dregeana* the first stems developed 34, 40 and 74 days after germination respectively.

Dioscorea quartiniana is the South African species with the widest distribution throughout Africa, i.e. northwards in the eastern parts up to Ethiopia and in the west from the northern parts of South West Africa northwards to Senegal (Burkill, 1939). Like other tropical species (Martin and Ortiz, 1963; Sarwa, 1971) the majority of *D. quartiniana* seedlings developed only one leaf, while the others developed two leaves and a short stem within the first growing season as in the other three species. When the stem first appeared, it could have been mistaken for a third leaf, that is until a swelling appeared in the middle of its apparent petiole. This swelling is the node bearing the third leaf, while the growing point is hidden in the sheath of that leaf.

Most of the seedlings of the four species, i.e. 69 out of 105 produced two basal leaves before the emergence of the rest of the primary shoot. Twenty-three seedlings developed only one basal leaf on the stem, the second leaf being reduced to a small bract. Each of the remaining seedlings developed two leaves and two stems, the second stem possibly developing from the axillary bud of one of these leaves. These observations confirmed those made on *D. elephantiipes* in which the first two leaves are the most basal leaves of the primary shoot (Sparshott, 1935). This seems acceptable because although leaves of adult plants are arranged alternately, opposite leaves are occasionally found at some of the basal nodes. The stem of the seedling usually dies off completely in the winter. During the next growing season the new stem develops from one of the hidden axillary buds of the basal leaves.

3.2 Development and morphology of the intermediary organs

Although initially all the tubers from the same lot of seedlings looked similar (Fig. 3F, G and H), a remarkable difference in shape could be distinguished once the seedlings were two months old.

In the one lot for example, 20 of the 30 seedlings had pear-shaped tubers (Fig. 4B), while the remaining seedlings had distinctly elongated cylindrical organs (Fig. 4C). In some seedlings the developing cylindrical organ tuberised entirely,

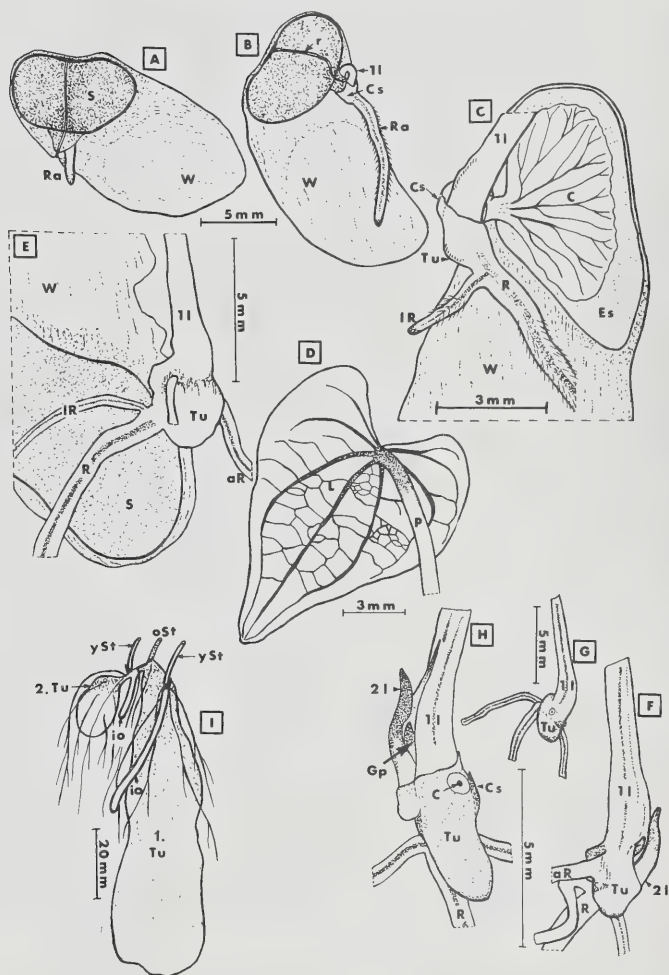


FIG. 3.

Tuber development of *D. cotinifolia*. A: radicle appears; B: first leaf appears; C: seed is dissected to show the cotyledon lamina; D: lamina of first leaf; E: displacement of main root by tuber growth; F, G, H: very young tubers; I: tuber of seedling at beginning of second season. *aR* adventitious root; *C* cotyledon; *Cs* cotyledonary sheath; *Es* endosperm; *Gp* growing point of stem; *io* intermediary organ (cylindrical); *lR* lateral root; *1l* first vegetative leaf; *2l* second leaf; *L* lamina; *oS* old stem; *P* petiole; *Ra* radicle; *R* main root; *r* raphe; *S* seed testa; *Tu* tuber; *W* seed wing; *ySt* young stem.

Subterranean intermediary organs of Dioscorea cotinifolia Kunth 49

thus also becoming pear-shaped. In other seedlings only the distal part tuberised while the proximal part produced a lateral branch which also formed a tuber. In any of these seedlings branches with swollen tips could also develop from the tuber crown, i.e. the proximal part of the tuber which gives rise to a stem each year. Seedlings found in the field (Fig. 4D) confirmed that these two types of tuber development were natural and not induced by experimental conditions. Our results

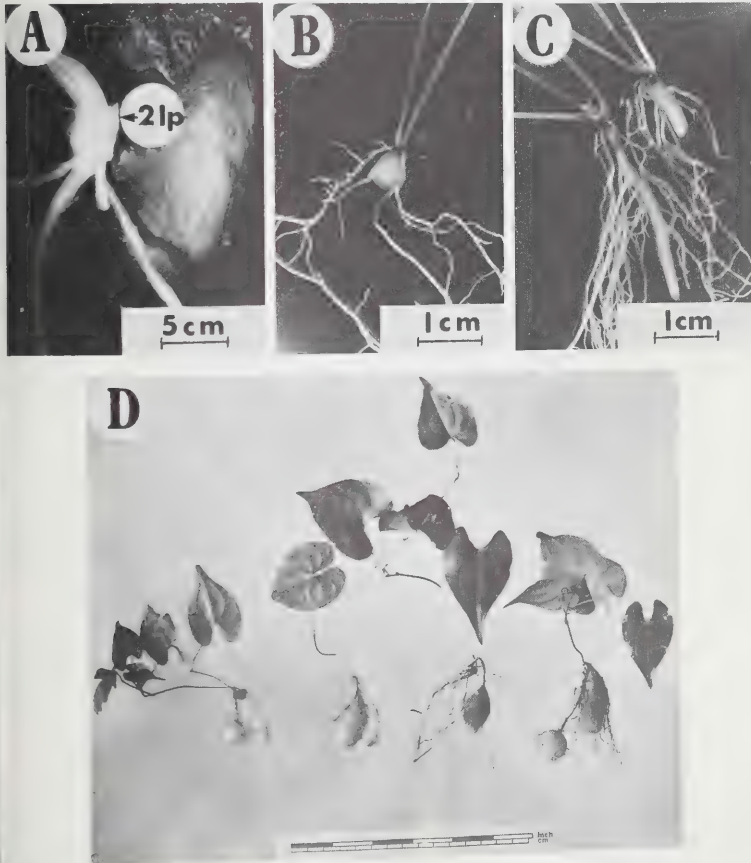


FIG. 4.

Developing tubers of *D. cotinifolia* seedlings. A: second leaf primordium (2lp) developing; B: seedling with a pear-shaped tuber and stem with one basal leaf; C: seedling with first cylindrical intermediary organ before tuberisation, the stem having two basal leaves; D: seedlings collected in the field illustrating tuberisation of the intermediary organs.

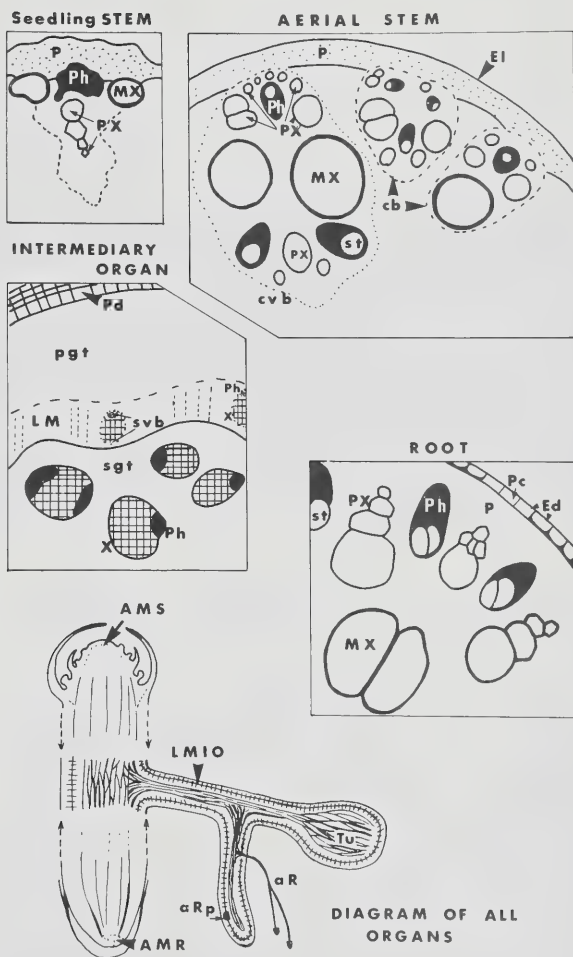


FIG. 5.

A diagrammatic representation of the position and anatomy of the different organs. *AMS* apical meristem of shoot; *AMR* apical meristem of root; *aR* adventitious root; *aRp* adventitious root primordium; *cb* common vascular bundle; *cvb* cauline vascular bundle; *Ed* endodermis; *El* endodermoid layer; *LM/LMIO* deep-seated lateral meristem of intermediary organ; *MX* metaxylem; *P* pericycle; *Pc* passage cell; *Pd* periderm; *pgt* parenchymatous or peripheral ground tissue; *Ph* phloem; *PX* protoxylem; *sgt* sclerenchymatous and/or central ground tissue; *st* sieve tube; *svb* secondary vascular bundle differentiating; *Tu* tuber; *X* xylem.

confirmed the observations of Archibald (1967) who wrote "Juvenile plants bear up to four thick roots* from 2–8 cm long, 2 mm diam, each terminating in a tuber 10–20 mm diam," but our interpretation differs from hers.

We regard the initially elongated, cylindrical organs, their branches, the crown and its branches and the resulting tubers as organs intermediate between a shoot and a root. They not only all originate from the hypocotyl but are also alike in their morphology. A shoot-like anatomical feature is the presence of closed, collateral vascular bundles (Fig. 5). Similar vascular bundles occur in the rhizome of *Rajania cordata* and *Dioscorea caucasica* (Ayensu, 1972) as well as in the seedling stem of *D. cotinifolia* (Fig. 5). The tubers are also similar to rhizomes in that they are perennial storage organs with adventitious roots. The presence of a periderm is a feature found in other monocotyledonous rhizomes, stems and roots (Tomlinson, 1969) and in *D. cotinifolia* a periderm is found in the intermediary organs as well as in the subterranean part of an adventitious stem (Fig. 6B and C). The apices of young, cylindrical intermediary organs have no root-caps, while older tips are covered by a periderm. The tuber tips of other *Dioscorea* species shows this too (Koch and Bruhn, 1962; Martin and Ortiz, 1963). The root-like anatomical features include the absence of a cuticle and stomata. Like roots, the very young intermediary organs have unicellular structures resembling root hairs which probably play a role in the uptake of water and nutrients. The cylindrical intermediary organs are organographically so similar to roots that they have been mistaken for roots (Codd, 1960; Archibald, 1967). As in roots the intermediary organs of *D. cotinifolia* never possess nodes, internodes or any signs of leaves. Von Guttenberg (1968) stated that, with a few exceptions, all monocotyledonous roots have an exodermis and endodermis, neither of which is present in *D. cotinifolia*'s intermediary organs. Unlike the tubers of *D. discolor* (Lindinger, 1907) and *D. batatas* var. *illustrata* (Von Guttenberg, 1968) the tubers of *D. cotinifolia* do not arise from roots.

The presence of a deep-seated lateral meristem, called a growth zone or cambium by other workers, is not too surprising in *Dioscorea* as it occurs in other monocotyledonous organs. Example: the stems of *Cordyline*, *Dracaena* and *Yucca*, where it forms secondary vascular bundles within a secondary ground tissue (Zimmermann and Tomlinson, 1972). This meristem is an outstanding characteristic of many Dioscoreaceae tubers but has never been found in their shoots or roots. In *D. cotinifolia* this meristem is found in the intermediary organs (Fig. 5) and gives rise to secondary vascular bundles, secondary ground tissue as well as lateral branches.

In a cultured seedling in its second growing season, young stems developed adventitiously from the original tuber as well as from a tuberised lateral branch of the crown (Fig. 3I). At the base of each young stem, adventitious roots as well as

*roots underlined by authors.

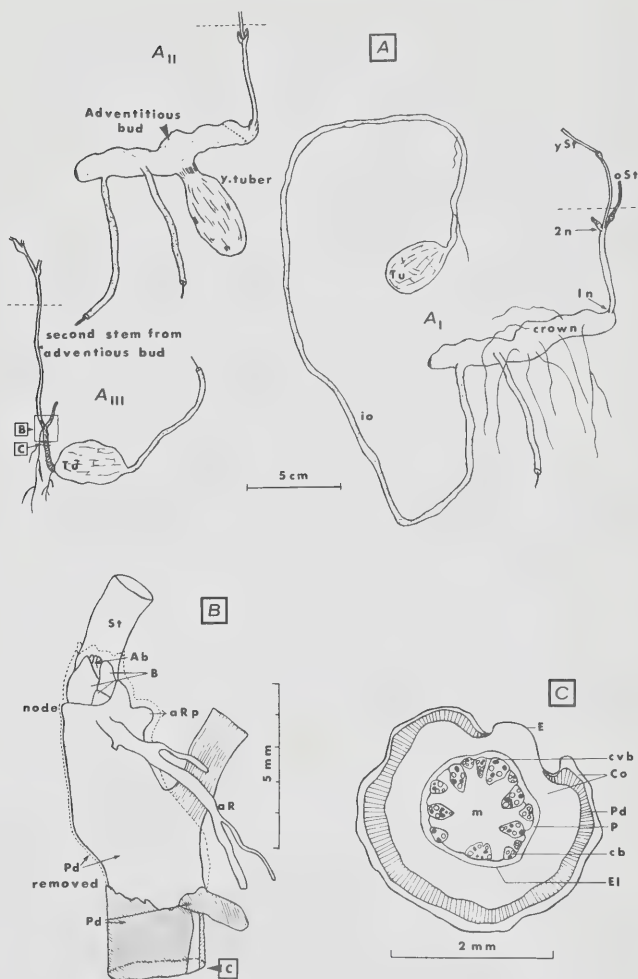


FIG. 6.

An adult plant of *D. cotinifolia* illustrating vegetative reproduction. A_I: tuber system as collected in the field; A_{II}: a part of A_I four months later; A_{III}: a part of A_I 12 months later; B: a part of the adventitious stem in A_{III}; C: Diagrammatic representation of the t.s. of the stem in B. Ab axillary bud; aR adventitious root; aRp adventitious root primordium; B bract; cb common vascular bundle; Co cortex; cvb cauline vascular bundle; E epidermis; El endodermoid layer; io intermediary organ; m pith; In & 2n first and second node; oSt old stem; p pericycle; pd periderm; St stem; Tu old tuber; ySt young stem; y-tuber young tuber.

two cylindrical intermediary organs developed. The latter became 18 and 25 cm long respectively and tuberised during that season. In other cultured seedling as well as one adult plant collected in the field two similar young adventitious stems developed from each plant. At the base of each young stem a new actively growing crown formed. The two new crowns in each plant remained active for two to four seasons. Generally the tubers of *D. cotinifolia* persisted for about three or four seasons as was also observed by Archibald (1967).

In the beginning of the growing season while the shoot developed, a number of adventitious roots up to 60 cm in length developed from the base of the new shoot. Some of the food reserves of the older tubers probably were utilised in this initial spurt of growth of the shoot. Later in the season existing younger tubers enlarged and tubers formed from newly formed cylindrical intermediary organs or from a thrust of growth from the crown (Fig. 6AI and AII) or from older cylindrical intermediary organs.

4 VEGETATIVE REPRODUCTION

Cylindrical intermediary organs of plants collected in the field were up to 3.3 m in length. Over a period of four seasons, some of these intermediary organs decayed or were broken accidentally during examination. The tubers which were separated from the original plant (e.g. Fig. 6AI) then formed adventitious shoots and developed into new plants. In the example illustrated in Fig. 6AIII, a periderm developed in the cortex of the subterranean part of the adventitious shoot (Fig. 6B and C). This is the first report of a periderm in the shoot of *D. cotinifolia*.

It seems very likely that tubers or intermediary organs can break off from the original plant in its natural habitat. Since all intermediary organs possess a deep-seated lateral meristem (Fig. 5) from which adventitious buds, roots or further intermediary organs can arise, any part of an intermediary organ which breaks off would be likely to form a new plant. This means of vegetative reproduction has been observed in the field by Miège (1950). He described how underground horizontal branches of *D. minutiflora* produced "callosities" and later separate plants at the nodes. In *D. minutiflora* even branches of the aerial shoot behave similarly when they fall to the ground accidentally. Martin and Ortiz (1963) reported the development of separate plants from partially buried stems, stem cuttings and tuber pieces in *D. spiculiflora* and *D. floribunda*.

5 CONCLUSIONS

Germination studies on *D. cotinifolia* indicate that the embryo has only one cotyledon and that: (1) two vascular traces enter the cotyledonary petiole while three enter the first vegetative leaf; (2) the venation of the lamina of these two leaves differs; and (3) the embryo has a minute first vegetative leaf.

It is, therefore, not always possible to classify organs as shoots or roots, and provision should be made, as was stated by Sattler (1974), for a category of organs which are intermediate between the two.

Dioscorea cotinifolia has a specialised tuber system. Our reasons for regarding the initially elongated, cylindrical organs, their branches, the crown and its branches and the resulting tubers as organs intermediate between a shoot and a root are the following: (1) they originate directly or indirectly by branching and tuberisation from the hypocotyl which, in the adult plant, represents the transition region which has "features that are intermediate or transitional between those of a shoot and the root" (Esau, 1967); (2) the growth of the intermediary organs results from the activity of a deep-seated lateral meristem which is neither found in shoots nor in roots of *Dioscorea*; and (3) these organs have characteristics typical of both the shoot and the root.

Besides functioning initially as absorptive organs, the intermediary organs bear adventitious roots which anchor the plant. The tubers serve mainly as storage organs while the cylindrical intermediary organs serve as organs of transport. Any of the intermediary organs may act as vegetative reproductive organs.

It would be interesting to study *D. arcuatinervis*, *D. mamillata* and *D. proteiformis* from the Malagasy Republic since their tubers are also borne at the end of long cylindrical organs (Burkill, 1950). These tubers might represent highly specialised structures just like those of *D. cotinifolia* and of *D. glabra* which are borne on a sympodial rootstock (Sharma, 1974).

ACKNOWLEDGEMENTS

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APPENDIX Nutrient solution used for seedlings:

<i>Macroelements</i> (gl ⁻¹)	
MgSO ₄ · 7H ₂ O	0,266
KCl	0,302
KH ₂ PO ₄	0,066
CaH ₄ (PO ₄) ₂ · H ₂ O	0,072
CaSO ₄ · 2H ₂ O	0,249
NH ₄ NO ₃	0,880
Ca(NO ₃) ₂ · 4H ₂ O	0,354

Microelements according to Hewitt (1952) p. 109: A-Z supplements as under A. MoO₂ - 0,024 mg l⁻¹ and Fe - 2 mg l⁻¹ using Na Fe. EDTA were also added.

REFERENCES

- ARCHIBALD, E. E. A., 1967. The genus *Dioscorea* in the Cape Province west of East London. *Jl S. Afr. Bot.* **33**: 1-46.
- AYENSU, E. S., 1972. Dioscoreales. In: C. R. Metcalfe, (ed.), *Anatomy of the Monocotyledons*. VI. Oxford: Clarendon Press.
- BECCARI, O., 1870. Nota sull'embrione delle Dioscoreacee. *Nuovo G. bot. ital.* **2**: 149-155.
- BUCHERER, E., 1889. Beiträge zur Morphologie und Anatomie der Dioscoreaceen. *Biblthca bot.* **3**(16): 1-35.
- BURKILL, I. H., 1939. Notes on the genus *Dioscorea* in the Belgian Congo. *Bull. Jard. bot. nat. Belg.* **15**: 345-392.
- BURKILL, I. H., 1950. Dioscoreae. In: H. Humbert, (ed.), *Flore de Madagascar et des Comores*. 44^e Famille. Paris: Typographie Firmin-Didot et Cie.
- BURKILL, I. H., 1960. The organography and the evolution of Dioscoreaceae, the family of the Yams. *J. Linn. Soc. (Bot.)* **56**: 319-412.
- CODD, L. E., 1960. Drugs from wild Yams. *Afr. wild Life* **14**(3): 215-226.
- DE BARY, A., 1884. *Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns*. Oxford: Clarendon Press.
- ESAU, K., 1965. *Plant Anatomy*. 2nd edition. New York: John Wiley & Sons, Inc.
- GOEBEL, K., 1905. Morphologische und biologische Bemerkungen. 16. Die Knollen der Dioscoreen und die Wurzelträger der Selaginellen, Organe, welche zwischen Wurzeln und Sprossen stehen. *Flora, Jena* **95**: 167-212.
- HEWITT, E. J., 1952. *Sand and water culture methods used in the study of plant nutrition*. Bucks, England: Commonwealth Agricultural Bureaux.
- KOCH, W. and BRUNN, C., 1962. Über die Morphologie der Speicherorgane einiger mittelamerikanischer Dioscoreen. *Flora, Jena* **152**: 670-678.
- LAWTON, J. R. S. and LAWTON, J. R., 1967. The morphology of the dormant embryo and young seedling of five species of *Dioscorea* from Nigeria. *Proc. Linn. Soc. Lond.* **178**: 153-159.
- LINDINGER, L., 1907. Über den morphologischen Wert der an Wurzeln entstehenden Knollen einiger *Dioscorea*-Arten. *Beih. bot. Zbl.* **21**(1): 311-324.
- MARTIN, F. W. and ORTIZ, S., 1963. Origin and anatomy of tubers in *Dioscorea floribunda* and *D. spiculiflora*. *Bot. Gaz.* **124**: 416-421.
- MIEGE, J., 1950. Caracteres du *Dioscorea minutiflora* Engl. *Revue int. Bot. appl. Agric. trop.* **30**: 428-432.
- RAO, A. N., 1953. Embryology of *Dioscorea oppositifolia* L. *Phytomorphology* **3**: 121-126.
- SADIK, S. and OKEREKE, O. U., 1975. Flowering, Pollen Grain Germination, Fruiting, Seed Germination and Seedling Development of White Yam, *Dioscorea rotundata* Poir. *Ann. Bot.* **39**(161): 597-604.
- SARWA, M., 1971. Growth and development of *Dioscorea spiculiflora* Hemsl. and Cross Fertility with *D. composita* Hemsl. *Angew. Bot.* **45**: 157-161.
- SATTIER, R., 1974. A New Conception of the Shoot of Higher Plants. *J. theor. Biol.* **47**: 367-382.
- SHARMA, O. P., 1974. Anatomy, origin and development of tuber of *Dioscorea glabra*. *Phytomorphology* **24**: 297-305.
- SMITH, P. M., 1916. The development of the embryo and seedling of *Dioscorea villosa*. *Bull. Torrey bot. Club* **43**: 545-558.
- SPARSHOTT, E. N., 1935. Observations on the formation, development, and structure of the tuber of *Testudinaria elephantipes*, and on the origin of the vegetative shoot. *J. Linn. Soc. (Bot.)* **49**: 593-610.

- TOMLINSON, P. B., 1969. Commelinales-Zingiberales. In: C. R. Metcalfe, (ed.), *Anatomy of the Monocotyledons*. III. Oxford: Clarendon Press.
- TROLL, W., 1967. *Vergleichende Morphologie der höheren Pflanzen*. 1 Band, 1 Teil, Autorisierter Nachdruck. Koenigstein-Taunus: Otto Koeltz.
- VON GUTTENBERG, H., 1968. Der primäre Bau der Angiospermenwurzel. In: W. Zimmermann *et al.*, (eds.), *Handbuch der Pflanzenanatomie*, Zweite Auflage, Spezieller Teil, Band 8, Teil 5. Berlin: Gebrüder Bornträger.
- VON TEICHMAN UND LOGISCHEN, I., 1973. 'n *Morfologiese ondersoek van die Transvaalse Dioscorea-soorte*. Unpublished M.Sc. thesis. Pretoria: University of Pretoria.
- VON TEICHMAN UND LOGISCHEN, I., VAN DER SCHIJFF, H. P. and ROBBERTSE, P. J., 1975. The Genus *Dioscorea* L. in South Africa. *Boissiera* **24**: 215-224.
- WEBER, H. and TROLL, W., 1969. Morphologie einschliesslich Anatomie. In: H. Ellenberg *et al.*, (eds.), *Fortschritte der Botanik* III. Band 31. Berlin: Springer-Verlag.
- ZIMMERMANN, M. H. and TOMLINSON, P. B., 1972. The vascular systems of monocotyledonous stems. *Bot. Gaz.* **133**: 141-155.